SENSITIVITY AND SPECIFICITY OF LAMP IN DETECTING MALARIA PARASITE INFECTION IN ENDEMIC AREAS OF BINH PHUOC PROVINCE IN 2017

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Summary

The study was conducted in two communes of the mesoendemic malaria area of Binh Phuoc province, using the giemsa technique and LAMP technique to detect malaria parasites. The purpose of the study was to determine the prevalence of malaria parasites in the community and to evaluate the sensitivity, specificity and predictive value of LAMP compare giemsa technique (gold standard). As a result, 384 samples of human blood were collected and analyzed: The prevalence of malaria parasitic by giemsa was 0.52% (2/384) and LAMP was 4.43 % (17/384). The sensitivity of the LAMP technique was 100% (02/02), the specificity was 96.07% (367/382). Positive predictive value was 11.76% (2/17), negative predictive value was 100% (367/367).

Key words: *Sensitivity /specificity, LAMP, malaria endemicity /Binh Phuoc.*

INTRODUCTION

LAMP technique (loop - mediated isothermal amplification) is a relatively new molecular biology technique that has been used in many fields, with high sensitivity, specificity, and low cost. Particularly for detecting malarial parasites, this technique has detection limit for malaria parasites is very low (from 0.2 - 2 KST/µL) and can be carried out in the field. Giemsa technique is still a gold standard in the diagnosis and prevention of malaria. However, this technique has some drawbacks: detection limit for malaria parasites is high (4 - 5 malaria parasites/lµl blood) leads to the detection of remission in people with low malaria parasites, especially in human living in severe malaria endemic areas, persistent; depending on the skill and qualifications of the examiner; depending on timeout when get blood to test. At present, molecular biology techniques such as Multiplex PCR, nested PCR, Real - time PCR are increasingly being used in the detection and identification of malaria parasites…

Thus, for the heavy prevalent malaria area previous, the current valid rate of malaria parasites were detected low that may be due to high levels of community- acquired immunosuppressive and "cold parasites", low malaria parasites in the body, the application of LAMP to evaluate the reality of malaria parasites infection in the community, thereby assessing the sensitivity, specificity of LAMP technique compared with current techniques - giemsa technique has important practical significance.

The study was conducted from 03/2017 to 12/2017 at the community of Thong Nhat and Dak Nhau communes of Bu Dang district, Binh Phuoc province where the heavy prevalent malaria area previous. Currently, in this community, malaria parasites have been detected every year, but the prevalence is much lower. In fact, due to the fact that only rapid test and giemsa technique are used in populations where malaria was heavy prevalent, the community may have a certain level of malaria immune response, the malaria parasite in the blood of a person may be very low, under the threshold of giemsa technique and rapid test.

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Therefore, with the use of LAMP technique in the study, the results may be more accurately reflected in the actual situation. The objectives of the study were to: (1) evaluate the reality rate of malaria parasites in the community; and (2) evaluate the sensitivity and specificity of LAMP technique in the diagnosis of malaria parasites.

MATERIALS AND METHODS

A cross - sectional survey was conducted in Thongnhat commune and Daknhau, Budang district, Binhphuoc province. Through random sampling method, data is collected from those who agree to blood tests look for Plasmodium by giemsa and LAMP techniques.

- Giemsa technique: Implement in according to the WHO guidelines.

- LAMP techniques: Extract DNA: (1) Heating: Incubate 1000 C/15 minutes; (2) Using DNA extraction kits from Thermo Scientific. Malaria parasite identification in 2 steps: Step 1. Detection of *Plasmodium* genus. Primer detection of *Plasmodium* genus: F3: 5'- GTA TCA ATC GAG TTT CTG ACC - 3', B3c: 5'- CTT GTC ACT ACC TCT CTT CT-3', FIP (F1c-F2): 5'- TCG AAC TCT AAT TCC CCG TTA CCT ATC AGC TTT TGA TGT TAG GGT - 3', BIP (B1 - B2c): 5'- CGG AGA GGG AGC CTG AGA AAT AGA ATT GGG TAA TTT ACG CG - 3', LPF: 5'- CGT CAT AGC CAT GTT AGG CC - 3', LPB: 5'- AGC TAC CAC ATC TAA GGA AGG CAG - 3'[3]. Reaction component: Isothermal master mix (OptiGene, UK): 15.0 µl, the total volume of 5.0µl (concentration: F3 & B3 5pM per primer, LPF & LPB 10pM per primer, FIP & BIP 20pM per primer), DNA template: 5.0 µl. Isothermal cycle: 65° C for 30 minutes. Step 2: Detection of *P. falciparum*, *P. vivax*. Positive specimens with *Plasmodium* genus will be identified species. Use of primer design software, reactive components and sothermal cycles as genus detecting. In detecting genus and species, the positive and negative samples were analyzed in parallel with the study sample. All reactions were implemented by Genie II of OptiGene, UK.

RESULTS AND DISCUSSIONS

Plasmodium prevalence at the study sites

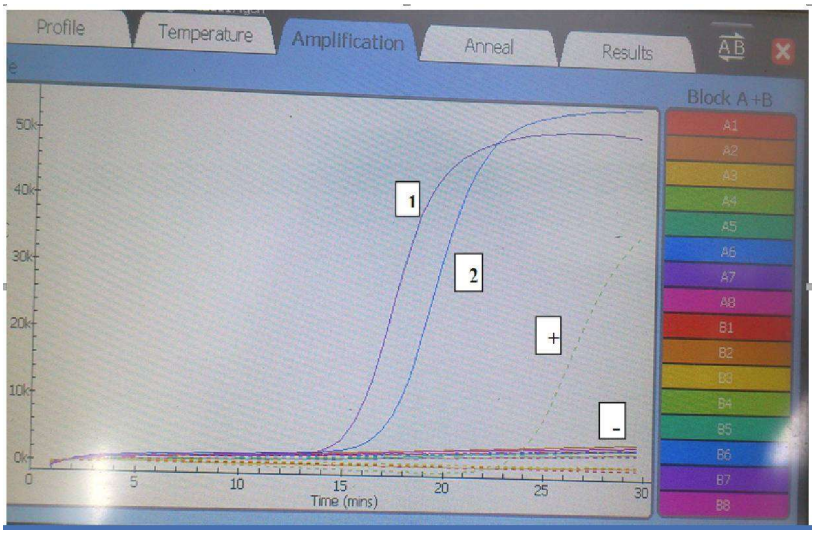
The analysis of 384 samples, results are shown in Table 1 as follows:

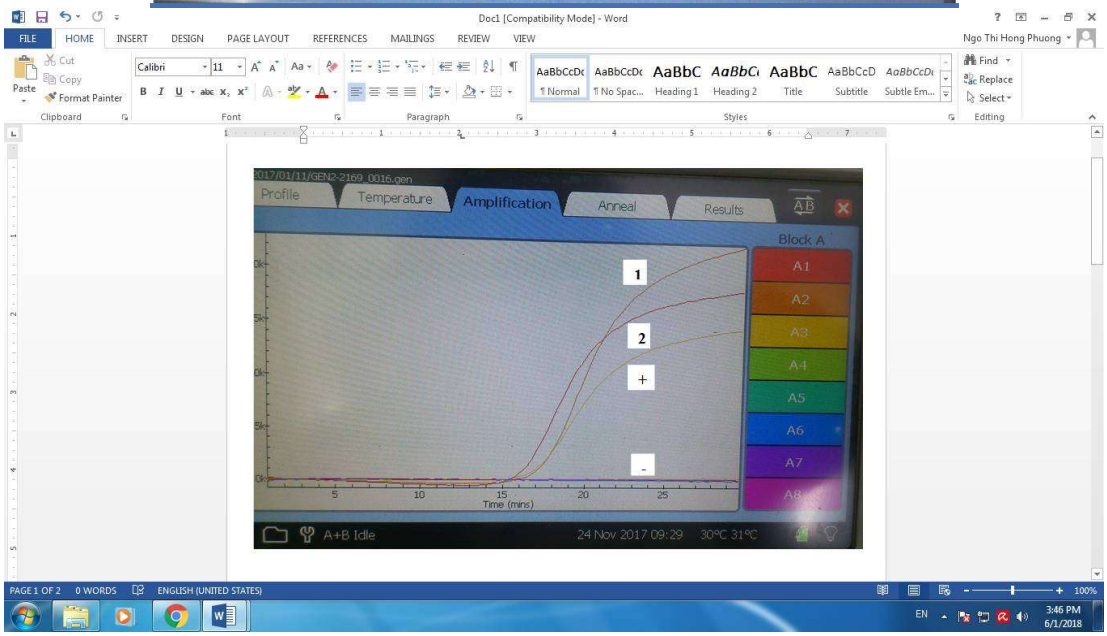
Table 1. Plasmodium prevalence at the study sites by giemsa and LAMP techniques, 2017

Study site	Sample	Giemsa		LAMP	
		Positive	Negative	Positive	Negative
Daknhau	240	0	240	7	233
Thongnhat	144	02	142	10	132
Total	384	02	382	17	367
Rate (%)		0.52	99.48	4.43%	95.57%
The difference is statistically significant (p = 0.0001)					
Species component	*P. falciparum*	02		02 (2/17)	
	P. vivax	0		0	

The rate of malaria parasites detected by giemsa technique was 0.52% (02/384). Both cases were detected to be *P. falciparum* infections. The rate of malaria parasites detected by LAMP technique was 4.43% (17/384), the difference is statistically significant (p = 0.0001) There are 2 cases of *P. falciparum* infection, accounting for 11.76% (02/17) in total cases that were detected positive with *Plasmodium* genus, 15 cases can not identify the species.

Figure 1 shows the results of analyzing sample for the detection of *Plasmodium* genus by LAMP technique, analysis samples at 1, 2 position shows the fluorescent signal appear at 15 - 16 minutes for 30 minutes of reaction, the fluorescent signal of analysis samples were higher than positive control and the melting temperature was 85ºC same with positive control. Figure 2 shows the results for detecting species. The sample of study site 1, 2 shows that the fluorescent signal appear at 14 minutes for 30 minutes of reaction, the fluorescent signal of analysis samples were higher than positive control and the melting temperature was 85ºC same with positive control. The fluorescent signal of negative control in both images were level zero.





Only 02 positive cases in all analysis samples were found by giemsa technique, accounting for 0.52% (02/384). Both cases were detecting to be *P. falciparum*. The study also performed rapid tests with these all samples and gave the results in the same giemsa technique. In comparison with the previous survey data, malaria parasite detected rates in this region with rapid test or giemsa technique are usually 7 - 15%[1]. LAMP analyzed 384 blood samples that tested rapid tests and giemsa technique, LAMP detected 17 positive cases with malaria parasites, accounting for 4.43%

(17/384). With the results of the LAMP technique show that malaria is still high present in Dak Nhau and Thong Nhat communes.

There are up to 15 cases or 3.91% of missed malaria parasites were not detected if performed by rapid and giemsa technique. This large difference may be due to the high malaria parasite detection of rapid test and giemsa technique, while the population of these two communes be long to in heavy prevalent area, persistent, may be with very low malaria parasite concentration in blood, below the detection threshold of rapid test and giemsa technique. Although malaria parasites are present in the blood but low density, the community may have an immune response to malaria and therefore may be clinically asymptom.

Compared to the results of testing with the Nested - PCR technique, at these two communes in 2015, when conducting the Active Case Detection study (ACD), the results showed that the positive rate for *Plasmodium* was 23.00%. 49/213)[4]. The difference in the rate detection of malaria parasites in the two studies was hight. About detecting limit malaria parasites of two techniques, with malaria detection limit of at least 6 malaria parasites/ μL of nested - PCR technique, while malaria detection limits for LAMP are 0.2 - 2 malaria parasites/μL. So, the LAMP technique must detect more malaria parasites cases than the nested - PCR technique of level 3 to 30 times, but the reality is less than 5 times lower. This disparity may be due to the fact that the rate of malaria parasites in the two communes has decreased sharply over the past two years, and thus shows that malarial activity in recent years has been highly effective at this area. This disparity may also be due to the problem of sampling, in a study using the Nested - PCR technique in 2015, blood samples were mainly collected from subjects suspected of being infected or exposed to malaria (forests, sleeping areas, people who have recently had or have had malaria, people from the malaria area...).

Sensitivity and specificity of LAMP technique in the diagnosis of malaria parasites.

The results of detecting malaria parasite by LAMP technique compared with the giemsa technique are presented in Table 2 for evaluating the sensitivity and specificity of LAMP technique:

Table 2. The results of LAMP technique in detecting of malaria parasitic compared with results of giemsa technique

		Results of Giemsa technique		Total
		Positive	Negative	
Results of LAMP technique	Positive	02	15	17
	Negative	0	367	367
Total		02	382	384

Sensitivity (or true positive rate) = 100% (02 / (2 + 0))

Specificity (or true negative rate) = 96.07% (367 / (367 + 15))

Positive predictive value (probability of an individual) = 11.76% (2 / (2 + 15).

Negative predictive value (probability of an individual not being sick) = 100% (367/(0 + 367)).

Giemsa technique detected two positive cases with *Plasmodium*, while analysed sensitivity for the LAMP show that sensitivity (true positive) of LAMP is high absolute (100%), this technique don't miss a case with malaria parasites compare result of rapid test and giemsa technique. However, LAMP also deteted 15 cases that were negative rapid test and giemsa technique. Therefore, rate of the false positive rate of LAMP was 11.76% (15/17), pretty hight. From 2 results of true positive and false positive rate, if the based of the giemsa technique, the positive rate with malaria parasite in community research is 0,52%, if take this value for attraction reality malaria parasite case in this community will not exact. So, with false positive rate will be calculated positive predictive value and show that probability of disease of a personal in this community is 11.76% (2/17). May be giemsa technique has low diagnostic value at the population in the heavy prevalent area, persistent, and as said abow may be the problem of the community of this two communes be long to heavy prevalent area, persistent, density of malaria parasite in the blood is very low, under detect limit the test of rapid test and giemsa technique.

Specificity (or true negative rate) in this research also very hight is 96.07% (367/382), negative predictive value is 100% (367/367), which is probability not sick of a person in this

community is 100%, this is only value when say about engineering, does not meaningfully in reality. Because rapid test and giemsa technique don't have confusion when test negative cases for positive results that can only be confusion from malaria positive case to negative cases (this is found at the false positive rate in this study).

This is a research on the population, when collect blood samples at the field, short time and collecting blood samples at work time so working age objects - who have risk of malaria infection were absent. Therefore does not accurately reflect the malaria positive cases rate in reality population, however, the main aim is valuating sensitivity, specificity of LAMP so can be restricted of the other tasks. Assume that if the rapid test or giemsa technique is chosen samples for the right size, composition and distribution of the sample (study population) selected for study were correct representation of the population, then the probability of malaria parasite infection Detected by rapid test and giemsa technique may be increased, with a positive predictive value increasing.

About to the sensitivity, specificity of the LAMP technique, this study show that sensitivity, specificity of LAMP technique is very high and has many of the author on the world that had reported sensitivity, specificity of LAMP technique at level 100%. Adding the malaria detection limits that WHO published[5] show LAMP technique is more preeminent than giemsa technique in detecting malaria parasites.

Through reference several author on the world about researching sensitivity, specificity of the LAMP technique as study of Mariko Yamamura et al. (year 2009)[2] show that LAMP technique have sensitivity is 97.8% and specificity is 100%, Yee Ling Ling et al. (year 2016)[5], the sensitivity and specificity is 100%, in this study show sensitivity is 100% and specificity is 96.07%. Result in this study was quite relative so with the results of present abow the research.

Extra 02 positive cases with *Plasmodium* that rapid test and giemsa detected, LAMP and real-time PCR had detected 15 positive cases with *Plasmodium*. According the published of WHO about detection limit of molecular biology techniques in diagnosing malaria parasite present detection limit of LAMP techniques is 0.2 - 2 malaria parasite/ µL blood, real - time PCR is 0.02 KST/µL blood, while limit of giemsa is 5 malaria parasite/ µL blood. According Yee - Ling Lau et al. (year 2016)[5] when comparing LAMP techniques and giemsa, Nested PCR, a composite diagnosis for each sample (two of three tests giving the same result) was created and used as a reference for all three test modalities.

Besides, from the results of the application LAMP displayed: LAMP detected 17 positive cases with *Plasmodium* but only detecting 02 positive cases with *P. falciparum*, other 15 cases don't detect *P. falciparum* or *P. vivax* species. In 17 positive cases with *Plasmodium*, this study also perform real - time PCR, detect 04 *P. falciparum*, 17 *P. vivax*. Fluorescent signal of 02 *P. falciparum* case same result of giemsa and LAMP were very high, fluorescent signal of other cases is very low and present at the last cycles. This show that concentrate DNA or concentrate malaria parasite in blood sample is very low. Follow the publication of WHO about detection limit of molecular biology techniques in diagnosing malaria parasite present detection limit of real - time PCR is 0.02 malaria parasite/µL blood in detection *Plasmodium* genus and 1.22 malaria parasite/µL detection *P. falciparum*. To detecting species requires concentrate malaria parasite in sample higher when detecting genus same as also in the PCR to detecting species the regular must run PCR 2 step as nested - PCR. LAMP technique also must use primers, enzyme to lengthening double DNA string, so may be when detecting species is this technique requires concentrate malaria parasite higher when detecting genus. Therefore, when detecting malaria parasite species by LAMP technique is this technique can't detect species of 15 cases and can't detect combined infections cases.

CONCLUSIONS

In conclusions, 384 samples of human blood were collected and analyzed to detect malaria parasites:

- The prevalence of malaria parasitic by giemsa was 0.52% (2/384) and LAMP was 4.43% (17/384).
- The sensitivity of the LAMP technique was 100% (02/02), the specificity was 96.07% (367/382).
- Positive predictive value is 11.76% (2/17), negative predictive value is 100% (367/367).

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